Middle Mississippi River Sturgeon Preliminary Contaminants Investigation

Illinois, Missouri and Iowa

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Abstract

We examined the health of forty-one wild caught sturgeon from a reach of the Mississippi River with an organochlorine consumption advisory and a reference site. The health indices included physical and microbiological examinations, endocrine disruption, histopathology and whole carcass contaminant analysis. All of the sturgeon from the sampling location within consumption advisory reach had relatively larger livers compared to the sturgeon from the reference site. We observed health anomalies in the male specimens from the sampling location within the consumption advisory reach. The anomalies included plasma estrogen and testosterone ratios that were greater than one for three male sturgeon specimens and vitellogenin was induced in two of these three cases. Two other male sturgeon specimens taken from the consumption advisory reach had intersex characteristics. These sturgeon specimens with the health anomalies ranked among the fish with the highest tissue chlordanes+PCB concentrations. The apparent toxicological effects threshold for chlordanes+PCB was greater than 1.2 μg/g ww whole body concentration, however there was an exception.

Introduction

Background

Previous assessments have identified a variety of contaminant concerns for sturgeon species in the Mississippi River Basin (UMRBA 1989 and UMRCC 1996). Human health consumption advisories have existed since the mid 1980's for sturgeon meat and roe along the middle reach of the Mississippi River (MODH 1991). The advisories are based on chlordane (an organochlorine insecticide) and polychlorinated biphenyls (PCBs). Sturgeon eggs from the Chester, Illinois reach (south of St. Louis) of the Middle Mississippi River had concentrations as high as 2780 micrograms of chlordane per kilogram, 5810 micrograms PCB per kilogram and 780 micrograms DDE per kilogram (Missouri Department of Health 1991). These contaminants are known to cause adverse health effects and mortality in fish (Jarvinen and Ankley 1999).

The Middle Mississippi River is also used by the federally listed endangered and closely related sturgeon, the pallid sturgeon (*Scaphirhynchus albus*). These two species of sturgeon are known to hybridize in the wild (Carlson *et al.* 1985, Henry and Ruelle 1992, Ruelle and Keenlyne 1994, and USFWS 1993). Pallid sturgeon may live longer and attain a larger size than the shovelnose sturgeon (Ruelle and Keenlyne 1994). In the modified river habitats, the two species may have similar spawning intervals and may use similar spawning locations (Ruelle and Keenlyne 1994). The shovelnose sturgeon feed primarily on aquatic insect larvae (Modde and Schmulbach 1977, Durkee *et al.* 1979, and Conte *et al.* 1988), while the pallid sturgeon feed primarily on fish and aquatic insects (Kallemeyn 1983).

Goal and Objectives

The goal of this study was to investigate the role of organochlorine chemical contamination has on limiting the recovery of the endangered sturgeon species along the Middle Mississippi River. This activity was identified as pallid sturgeon endangered species recovery plan objective 2.5 (USFWS 1993). The results from this preliminary study will be used to screen for problems and decide if the investigation should continue.

The objectives for the study were to :

1. Measure the organochlorine contaminant body burden in a group of the surrogate species, shovelnose sturgeon, taken from within the consumption advisory reach of the Middle Mississippi River and in a group from outside of the problem area.

2. Compare indices (biomarkers) of adult fish health between these two test groups.

Methods

The investigation focused on biomarkers of adult health. The fish biomarkers evaluated included physical condition, disease, histopathology, endocrine disruption and contaminant body burden.

Field Collections

The sturgeon were collected by commercial fishermen using gill nets suspended off of the bottom of the river at a location near Chester, IL (hereby known as the affected area) and from a reference site in Davenport, IA (Figure 1).

Physical Examination

An external and internal examination was completed following netting operations and various tissues and fluids were saved for analysis. Pre-determined observations were checked on each fish and noted on necropsy field sheets developed by the U.S. Geological Survey (Appendix A). Total fork length (to the nearest millimeter) and weight (to the nearest gram) were measured. Liver weight with gall bladder intact (to nearest 0.1 gram) was measured and compared to total weight (somatic index) as an indication of stress from contaminant exposure (Schmitt and Dethloff 2000). An attempt was made to process ten male and ten female fish from each site based on gross inspection of gonads and the number of fish retrieved by the fishermen.

Microbiology

Fish health specialists from the U.S. Fish and Wildlife Service's Fish Health Center, Onalaska, Wisconsin assisted in the physical examinations and they collected gastrointestinal tract, kidney and liver swabs for microbiological tests at the Fish Health Center. The microbiological tests included bacteria culture, viral culture and parasite examination.

Samples for bacteriology were collected by streaking kidney tissue onto Cytophaga Agar and Brian Heart Infusion Agar (BHIA) slants. The slants were transported to the Fish Health Center for bacterial isolation and screened for the following pathogens: *Aeromonas hydrophila* (Motile Aeromonad Septicemia), *A. salmonicida* (Furunculosis), *E. tarda* (*E. tarda* Septicemia), *Flexibacter columnaris* (Columnaris Disease), and *Yersinia ruckeri* (Enteric Red Mouth).

Samples for virology were collected by placing a small amount of kidney and spleen tissue from each fish into Hank's Balanced Salt Solution (HBSS) (samples

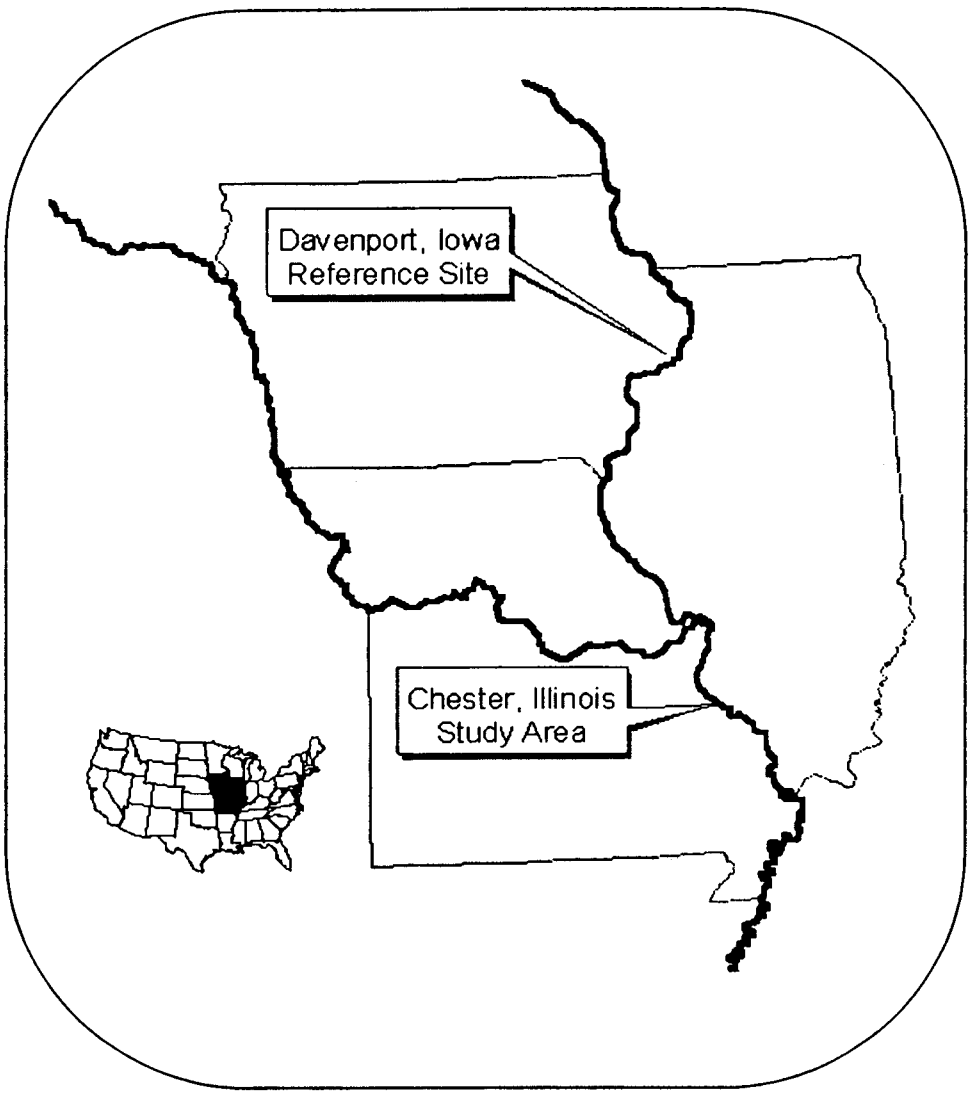

Figure 1. Locations of shovelnose sturgeon sampling sites along the Mississippi River, Illinois, Iowa and Missouri.

were combined into 5-fish pools). The samples were transported to the Fish Health Center for virology. Samples were incubated with white sturgeon spleen (WSS) and chinook salmon embryo (CHSE-214) cells for 14 days at 20°C and 15°C, respectively. Samples were presumed negative for viruses if cytopathic effect (CPE) was not observed after 14 days incubation

For parasite examination, the gastrointestinal (GI) tract was removed from each fish and placed into a plastic bag. Samples were then frozen with 95% ethanol that had been super-cooled with dry ice. Gastrointestinal tracts were transported to Fish Health Center on dry ice and stored at -20° C. Gastrointestinal tracts were then necropsied to determine the parasite load and diversity at each site.

Laboratory procedures followed the American Fisheries Society Bluebook: Suggested Procedures for the Detection and Identification of Finfish and Shellfish Pathogens (Thoesen 1994.).

Endocrine Chemistry

Whole fresh blood was collected from the caudal vein using a three cubic centimeter syringe and 21 gauge 1.5 inches (3.8 centimeters) needle that contained lining of heparin. The blood sample was dispensed into heparinized five millimeter vacutainers. The blood sample was chilled on wet ice until centrifuged in the field for 10 minutes at 1000 X *g* (where *g* is the acceleration due to gravity). The plasma sample was pipetted into two milliliter cyrotubes and frozen in 95% ethanol that had been super-cooled with dry ice. All blood samples were shipped following field activities to the U.S. Geological Survey's Fish Contaminants Science Center, Gainsville, Florida where they were stored at -80° C until analyzed.

Plasma samples were analyzed for estrogen and testosterone concentrations by radioimmunoassay procedures. Plasma samples were analyzed for vitellogenin concentrations by capture Enzyme-Linked Immunosorbent Assay (ELISA) procedures.

The ratio of total estrogen concentration and testosterone concentration (E/T) was used as an indicator of possible endocrine disruption (Goodbred *et al.* 1997). Male E/T ratios below 1.0 and female ratios greater than 1.0 was considered as normal for this study. A formal classification for this procedure has not been established. The presence of vitellogenin in the blood of male sturgeon was also be used as an indicator of possible endocrine disruption (Goodbred *et al.* 1997). The protein vitellogenin is used in egg production and has no known function in males, although other studies have documented the presence of vitellogenin at low concentrations in fish from minimally contaminated sites (Goodbred *et al.* 1997).

Histopathology

A one centimeter cross section each of liver and gonadal tissue from both sides of the body were taken and fixed in ten percent buffered neutral formalin solution. The formalin fixed tissues were transferred to the Registry of Tumors in Lower Animals at the Washington University Medical Center, Washington, D.C. The samples were embedded in paraffin, sectioned to 5 micrometers and stained with hematoxylin and eosin for histopathological examination. The histological examination included classifying the stage of sexual maturity for the gonads. The histological samples are achieved at the Registry.

Tissue Residue Chemistry

After necropsy, the remaining internal organs and tissues were placed back in the carcass cavity and wrapped in acetone rinsed aluminum foil. The dissected specimen was frozen in the field using dry ice. The specimens were stored until the chemical extraction process in a standard chest freezer. Ten carcasses from each site were randomly chosen for chemical analysis and forwarded to the contract laboratory under dry ice.

The carcasses were analyzed for lipid content, percent moisture and 22 organochlorine compounds including total polychlorinated biphenyls (PCBs) by the Mississippi State Chemical Laboratory, Mississippi State, Mississippi using standard methodologies. All values are reported on a microgram per gram wet weight basis (μg/g ww) with detection limits of 0.01 μg/g for all chemicals except for total PCBs, which had a detection limit of 0.05 μg/g.

Quality Control and Quality Assurance

Work surfaces and dissection instruments were decontaminated with potable water, acetone and final deionized water rinses. The performance of the field electronic balance was checked with a standard calibration weight at the beginning and end of each use.

Quality assurance testing for the endocrine chemistry was performed by the contract laboratory. These tests included analysis of known amounts of hormones.

Quality assurance testing for the tissue residue chemistry was performed by the contract laboratory. These tests included procedural blanks, spike recoveries, laboratory duplicates, data validation and report of anomalies by an outside chemist (Appendix B).

Data Management and Analysis

Field data, observations and instrument readings were recorded on special field sheets generated for this study (Appendix A). A laboratory analytical report with data validation is issued by the U.S. Fish and Wildlife Service Patuxent Analytical Control Facility (PACF) (Appendix B). The original field data sheets and laboratory report are archived in project files maintained at the Rock Island Field Office, Rock Island, Illinois.

The data from the field sheets and laboratory reports were entered into spreadsheets (Microsoft Excel). The data were graphically and statistically analyzed with Sigma Stat (Jandel Scientific).

Photograph © U.S. Fish and Wildlife Service

Results

Field Collection

Twenty-four sturgeon were examined at the Davenport, Iowa reference site on October 29, 1997, including what appeared to be 11 females and 13 males. Seventeen sturgeon were examined at the affected area on November 20, 1997, including what appeared to be 12 females and 5 males. Table 1 contains information if the specimen was analyzed for organochlorine chemicals, volume of sera collected for endocrine chemistry, length, weight and results of field determination of gender.

Physical Examination

Liver weight with gall bladder intact, liver to carcass weight ratio, carcass lipid content and carcass percent moisture data are listed in Table 2. The livers from the affected area group had a significantly greater relative weight compared to the reference site group (*t* Test p=0.000328).

Three individuals from the affected area contained a stomach content bolus of burrowing mayfly nymphs (*Hexagenia* and/or *Pentagenia* species). There were no other remarkable necropsy observations to note. The completed field necropsy sheets are in Appendix A.

Microbiology

Growth was observed on 79% (19/24) of the BHIA slants collected from reference site group, and on 94% (16/17) of the BHIA slants collected from the affected area group. None of the target bacterial pathogens (*A. hydrophila, A. salmonicida, E. tarda, F. columnaris, Y.ruckeri*) were observed at either site.

CPE was not observed in samples from either location after 14-d incubation with WSS and CHSE-214 cell lines, indicating that no viruses were detected. However, a virus may have been present but not observed due to the cell lines used.

There were no significant parasitology findings for the gastrointestinal examinations to report.

Table 1a. Characteristics of sturgeon specimens collected from the reference site.

Reference Number	Carcass Analyzed	Sera Volume (milliliter)	Total Length (millimeter)	Weight (gram)	Field Gender
Davenport, Iowa Reference Site					
U-01	No	1.0	450	275	F
U-02	Yes	2.0	500	392	M
U-03	No	0.1	437	260	F
U-04	No	1.0	462	325	M
U-05	No	1.0	550	550	M
U-06	No	None	487	350	F
U-07	No	0.05	437	280	F
U-08	No	None	510	460	M
U-09	Yes	2.0	505	450	M
U-10	Yes	1.6	552	635	F
U-11	No	1.8	467	370	M
U-12	Yes	1.8	612	820	F
U-13	Yes	2.2	490	375	M
U-14	Yes	2.2	537	657	F
U-15	No	1.2	510	485	F
U-16	Yes	2.2	577	800	F
U-17	No	1.0	487	357	F
U-18	Yes	1.8	702	1422	F
U-19	No	1.2	452	340	M
U-20	No	0.05	500	458	M
U-21	No	1.0	515	460	F
U-22	Yes	2.0	582	779	M
U-23	No	1.8	517	518	M
U-24	Yes	2.2	471	380	M
Mean			512.87	508.25	
SD[1]			61.11	254.22	

[1] SD = Standard Deviation

Table 1b. Characteristics of sturgeon specimens collected from the affected area.

Reference Number	Carcass Analyzed	Sera Volume (milliliter)	Length (millimeter)	Weight (gram)	Field Gender
Chester, Illinois Affected Area					
D-01	No	1.0	620	762	M
D-02	No	1.5	Missing data	588	M
D-03	Yes	1.8	625	734	F
D-04	Yes	1.8	645	752	F
D-05	No	1.8	745	1883	F
D-06	Yes	2.0	630	460	F
D-07	Yes	3.0	540	351	F
D-08	Yes	1.8	668	686	M
D-09	No	1.5	590	512	F
D-10	Yes	2.0	580	525	M
D-11	No	1.5	650	591	?
D-12	No	1.8	630	770	M
D-13	Yes	1.6	670	470	F
D-14	Yes	1.9	665	1328	M
D-15	Yes	1.3	595	746	F
D-16	No	1.0	530	335	F
D-17	Yes	2.0	655	1179	M
Mean			627.87	744.37	
SD[1]			54.85	404.87	

[1] SD = Standard Deviation

Table 2a. Specimen liver (gall bladder intact) weight, carcass percent lipid content and carcass percent moisture content for sturgeon collected from the reference site.

Reference Number	Liver Weight (gram)	Liver Weight / Carcass Weight	Carcass Percent Lipid	Carcass Percent Moisture
Davenport, Iowa Reference Site				
U-01	2.526	0.009185		
U-02	2.470	0.006301	1.20	76.5
U-03	1.672	0.006431		
U-04	3.010	0.009262		
U-05	2.09	0.0038		
U-06	3.492	0.009977		
U-07	2.03	0.00725		
U-08	4.73	0.010283		
U-09	4.062	0.009027	6.16	72.5
U-10	6.52	0.010268	13.3	68.5
U-11	3.414	0.009227		
U-12	4.792	0.005844	7.27	68.5
U-13	3.29	0.008773	5.32	74.5
U-14	7.68	0.011689	13.6	66.0
U-15	5.27	0.010866		
U-16	6.58	0.008225	14.4	64.5
U-17	3.384	0.009479		
U-18	14.0	0.009845	14.5	61.5
U-19	2.68	0.007882		
U-20	6.21	0.013559		
U-21	4.444	0.009661		
U-22	8.458	0.010858	8.07	70.0
U-23	4.078	0.007873		
U-24	3.512	0.009242	3.78	75.5
Mean	4.599	0.00895	8.76	69.8
SD[1]	0.550	0.000421	1.53	1.56

[1] SD = Standard Deviation

Table 2b. Specimen liver (gall bladder intact) weight, carcass percent lipid content and carcass percent moisture content for sturgeon collected from the affected area.

Reference Number	Liver Weight (gram)	Liver Weight / Carcass Weight	Carcass Percent Lipid	Carcass Percent Moisture
Chester, Illinois Affected Area				
D-01	17.0	0.02231		
D-02	11.5	0.019558		
D-03	15.6	0.021253	7.94	69.0
D-04	13.1	0.01742	6.30	69.0
D-05	27.8	0.014764		
D-06	10.5	0.022826	10.3	68.0
D-07	18.8	0.053561	13.5	67.5
D-08	10.4	0.01516	1.09	80.5
D-09	12.0	0.023438		
D-10	10.1	0.019238	9.19	67.5
D-11	15.1	0.02555		
D-12	18.3	0.023766	9.41	72.0
D-13	11.5	0.024468		
D-14	12.0	0.009036	4.63	75.0
D-15			12.9	65.5
D-16	11.3	0.033731		
D-17	17.3	0.014673	8.96	72.0
Mean	14.51	0.022547	7.83	71.4
SD[1]	1.155	0.002508	1.21	1.51

[1] SD = Standard Deviation

Endocrine Chemistry

The endocrine chemistry data are presented in Table 3. There were not statistically significant differences in the concentrations of male and female hormones between the affected area and reference site groups for fish of the same gender. The power of the performed statistical tests were below the desired power of 0.80 and the negative findings should be interpreted with caution.

There were three males from the affected area with an E/T ratio greater than unity and in two of these cases vitellogenin was detected in the blood (Table 3). Vitellogenin was detected in the blood of three males from the reference site. There were no female specimens with E/T ratios less than one.

Histopathology

There was no liver or gonadal neoplasms found. The livers of specimens from the reference site group appeared to be fattier compared to the affected area group, but this was not quantitated. The liver of most of the fish from both locations exhibited bile duct dilation, periductular fibrosis and perivascular inflammation, reminiscent of catfish with biliary myxidiosis. *Myxidium* species, was confirmed in bile ducts of one fish. A helminth parasite was seen in the liver of another fish.

Gonads from the reference site group were divided into 11 males and 11 females, two samples did not include gonadal tissue (Table 3). Following the staging of gonadal development listed by Van Eenennaam and Doroshov (1998), nine males were State 1 (testicles consisted of clusters of primary spermatogonia) and two were State 5 (testicles were filled with spermatozaoa). Nine females were Stage 2 (late regressed) and two were Stage 3 (early vitellongenesis).

Gonads of the affected area group were divided into seven males and 10 females (Table 3). Two males were immature but one was beginning maturation. One male was Stage 1, one was Stage 3 (onset of meiosis), two were Stage 4 (mid spermatogenesis) and three were Stage 5. Nine females were Stage 2 and one was Stage 4 (mid vitellongenesis).

The principal finding was two intersex male fish from the affected area. The testes of two of the Stage 5 male fish contained ovigerous lamellae in addition to mature sperm (reference numbers D-08 and D-11). In one of these fish (D-08), stalks of ovigeous lamellae extended from the tunica albuginea (Figure 2) and an island of ova was also present within the testes. In the other fish (D-11), ovigerous lamellae were only seen adjacent to the tunica albuginea. Since "sturgeons are gonochoristic and intersexes are rare and unusual" (Van

Eenennaam & Doroshov, 1998), these preliminary findings of intersexes are significant (Harshbarger 1999). It is possible other intersex fish were present in each group, but went undetected because we only examined one thin cross section of each gonad.

Tissue Residue Chemistry

Ten organochlorine chemicals out of the 22 tested were not detected at either of the locations: alpha BHC, beta BHC, delta BHC, endrin, gamma BHC, HCB, mirex, o,p'-DDD, o,p'DDE and toxaphene. Eight organochlorine chemicals were detected in the reference site group and 12 organochlorine chemicals, the same eight plus four more, were detected in the group from the affected area (Table 4) . The 12 organochlorine chemicals detected include (an asteric indicates that the chemical was common to both locations): alpha chlordane*, gamma chlordane, dieldrin*, epoxide mirex*, oxychlordane*, DDD*, DDE*, PCB*, cis-nonachlor and trans-nonachlor*. Note that out of the eight chemical types common to both locations only in six of these was the chemical detected in more than three fish from either group. Of these six chemicals common to most fish from each location, five pollutants were significantly different between the reference site and affected area groups (Table 4).

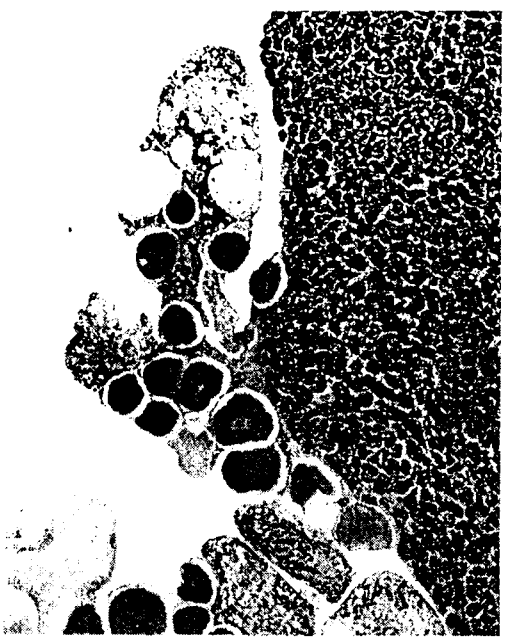

Figure 2. A stalk of ovigerous lamellae containing large oocytes and terminal follicular atresia extends from the tunica albuginea of a mature testes in a shovelnose sturgeon from the Middle Mississippi River, Illinois. 30 X (Harsharger 1999).

Table 3a. Plasma sample total estrogen, testosterone (picagrams per milliliter) and vitellogenin concentrations (milligrams per milliliter), ratio of estrogen and testosterone (E/T) and true gender for sturgeon specimens collected at the reference site.

Reference Number	Estrogen	Testosterone	Ratio E/T	Vitellogenin	True Gender
Davenport, Iowa Reference Site					
U-01	1362	1348	1.010385757	9.262	F
U-02	1086	830	1.308433735	0	M
U-03	1623	717	2.263598326	7.138	F
U-04	937	466	2.010729614	0	M
U-05	792	925	0.856216216	0	M
U-06	1335	791	1.687737042	2.877	F
U-07	950	290	3.275862069	10.202	F
U-08	711	1211	0.587118084	0	M
U-09	101	332	0.304216867	0	M
U-10	624	245	2.546938776	1.092	F
U-11	397	692	0.573699422	0	M
U-12	192	164	1.170731707	4.265	F
U-13	355	186	1.908602151	0	M
U-14	475	151	3.145695364	1.286	F
U-15	531	102	5.205882353	0.489	F
U-16	331	71	4.661971831	2.192	F
U-17	581	215	2.702325581	0.287	F
U-18	162	33	4.909090909	5.171	F
U-19	407	308	1.321428571	0.004	M
U-20	585	440	1.329545455	0.012	M
U-21	543	110	4.936363636	3.897	F
U-22	554	1289	0.429790535	0	M
U-23	635	1273	0.498821681	0.039	M
U-24	631	702	0.898860399	0	M
Male (n=12)					
Mean	599.25	721.17	1.002289	0.004583	
SD [1]	268.15	390.13	0.571891	0.011397	
Female (n=12)					
Mean	725.75	353.08	3.126382	4.013167	
SD [1]	481.98	396.52	1.501237	3.356354	

[1] SD = Standard Deviation

Table 3b. Plasma sample total estrogen, testosterone (picagrams per milliliter) and vitellogenin concentrations (milligrams per milliliter), ratio of estrogen and testosterone (E/T) and true gender for sturgeon specimens collected at the affected area.

Reference Number	Estrogen	Testosterone	Ratio E/T	Vitellogenin	True Gender
Chester, Illinois Affected area					
01	1230	1761	0.69846678	0	M
02	632	1301	0.485780169	0	F
03	670	128	5.234375	1.104	F
04	763	237	3.219409283	0.582	F
05	790	138	5.724637681	1.458	F
06	380	110	3.454545455	1.54	F
07	362	141	2.567375887	1.958	F
08	661	1211	0.545829893	0	I[1]
09	543	177	3.06779661	0.008	F
10	442	701	0.630527817	0	M
11	472	283	1.667844523	0	I
12	696	513	1.356725146	0.017	M
13	424	339	1.250737463	0.018	F
14	341	231	1.476190476	0.061	M
15	504	309	1.631067961	3.283	F
16	194	39	4.974358974	6.4	F
17	316	809	0.390605686	0	M
Male (n=5)					
Mean	605.00	803.00	0.910503	0.015600	
SD[2]	380.56	578.68	0.477691	0.026425	
Female (n=10)					
Mean	526.20	291.90	3.161008	1.635100	
SD	190.74	366.27	1.756153	1.968971	
Male Intersex (n=2)					
Mean	566.5	747	1.106837	0	
SD [1]	133.6432	656.1951	0.793384	0	

[1] Intersex
[2] SD = Standard Deviation

Table 4. Concentrations (milligrams per kilogram wet weight basis) of organochlorine chemicals detected in whole carcass sturgeon.

	alpha chloradane	gamma chloradane	oxy-chloradane	PCB Total	dieldrin	heptaclor epoxide	o,p'-DDT	p,p'-DDD	p,p'-DDE	p,p'-DDT	cis-nonachlor	trans-nonachlor
Davenport, Iowa Reference Site												
U-02	<.0100	<.0100	<.0100	0.094	<.0100	<.0100	<.0100	<.0100	0.021	<.0100	<.0100	<.0100
U-09	<.0100	<.0100	<.0100	0.22	0.016	<.0100	<.0100	<.0100	0.03	<.0100	<.0100	<.0100
U-10	0.013	<.0100	<.0100	0.2	0.1	0.02	<.0100	0.016	0.04	<.0100	<.0100	0.01
U-12	0.014	<.0100	<.0100	0.21	0.065	<.0100	<.0100	0.018	0.045	<.0100	<.0100	0.015
U-13	<.0100	<.0100	<.0100	0.16	0.014	<.0100	<.0100	<.0100	0.021	<.0100	<.0100	<.0100
U-14	<.0100	<.0100	<.0100	0.23	0.05	<.0100	<.0100	0.012	0.026	<.0100	<.0100	<.0100
U-16	0.013	<.0100	<.0100	0.49	0.06	0.012	<.0100	0.012	0.04	<.0100	<.0100	0.014
U-18	0.017	<.0100	0.013	0.28	0.11	0.023	<.0100	0.021	0.042	<.0100	<.0100	0.02
U-22	0.017	<.0100	<.0100	0.31	0.044	<.0100	<.0100	0.015	0.05	<.0100	<.0100	0.015
U-24	<.0100	<.0100	<.0100	0.14	<.0100	<.0100	<.0100	<.0100	0.017	<.0100	<.0100	<.0100
Mean	0.015		0.013	0.233	0.057	0.0183		0.0157	0.033			0.015
SD[1]	0.002		8.0E-307	0.110	0.035	0.0057		0.0035	0.012			0.004
Chester, Illinois Affected Area												
D-03	0.04	0.032	0.016	0.86	0.047	<.0100	0.012	0.034	0.1	0.012	0.03	0.072
D-04	0.036	0.031	<.0100	0.9	0.039	<.0100	0.013	0.04	0.12	0.013	0.039	0.082
D-06	0.036	0.027	<.0100	0.5	0.056	<.0100	0.01	0.029	0.075	<.0100	0.029	0.058
D-07	0.035	0.03	<.0100	0.61	0.081	<.0100	<.0100	0.037	0.068	<.0100	0.024	0.058
D-08	0.013	0.01	<.0100	1.2	<.0100	<.0100	0.022	0.019	0.17	<.0100	0.021	0.063
D-10	0.022	0.017	<.0100	0.31	0.055	<.0100	<.0100	0.014	0.053	<.0100	0.015	0.039
D-12	0.026	0.021	<.0100	0.75	0.054	<.0100	0.026	0.02	0.21	<.0100	0.02	0.049
D-14	0.057	0.048	0.015	1	0.048	<.0100	0.031	0.066	0.22	0.02	0.051	0.13
D-15	0.04	0.036	<.0100	0.45	0.11	0.017	0.015	0.031	0.07	0.019	0.021	0.054
D-17	0.1	0.083	<.0100	1.5	0.082	<.0100	0.019	0.078	0.21	0.013	0.073	0.18
Mean	0.041	0.033	0.015	0.808	0.063	0.017	0.018	0.037	0.130	0.015	0.032	0.078
SD[1]	0.024	0.020	0.0007	0.364	0.023	8.0E-307	0.007	0.020	0.066	0.004	0.018	0.044
Difference[2]	0.012			<0.001				0.011	<0.001			0.003

[1] SD = Standard Deviation

[2] Indicates a statistical significant difference between reference site and affected area data determined by either *t* Test or Mann-Whitney Rank Sum Test (*p* value).

Discussion and Conclusions

These findings suggest that pallid sturgeon health may be adversely affected by pollution in the Middle Mississippi River. A similar finding exists for pallid sturgeon in the Missouri River system (Ruelle and Keenlyne 1993). Endangered species managers should consider pollution in the home range of the pallid sturgeon species as one of the factors that may limit recovery of the species. Users of these results need to balance the weight-of-evidence that a problem exists as presented in this investigation against the study's conservative assumptions.

The results indicated that exposure to toxic contaminants in the Middle Mississippi River sturgeon caused health problems as evident by the observed anomalies for the biomarkers in fish from the affected area compared to no health anomalies observed in the reference site fish. The health problems were manifested in the male specimens of the affected area group (reference numbers D-08, D-12, D-14 and D-11). The sturgeon with the health anomalies ranked among the fish with the highest tissue chlordanes+PCB concentrations (1.22 to 1.683 µg/g ww) out of the total number of male fish tested. The carcass for one of the male specimens with health anomalies was not analyzed for contaminants (D-11). There was one male specimen (D-17) with the highest concentration of chlordanes+PCB (1.683 µg/g ww) that did not show any health anomalies. The apparent toxicological affects threshold for chlordanes+PCB was greater than 1.2 µg/g ww whole body concentration, however there was the exception of D-17.

The affected area group had apparently enlarged livers (25 times) based on relative liver size for the reference site group indicating stress from exposure to contaminants. The observed endocrine system anomalies included plasma E/T values greater than one (1.36, 1.47 & 1.68) and vitellogenin (0.017 & 0.061 milligrams per milliliter in plasma) was induced in two of these three cases. However, trace concentrations (<0.039 milligrams per milliliter) of vitellogenin was detected in the plasma of three fish from the reference site. Male vitellogenin may not be a preferred biomarker for sturgeon health assessments.

Two intersex male sturgeons out of the seven male specimens (29%) were observed in the affected area group. This is significant since "sturgeons are gonochoristic and intersexes are rare and unusual" (Van Eenennaam & Doroshov, 1998). The rate of intersex sturgeon may be higher than what was detected because only one thin section of gonad tissue from each testis was examined. Although other studies have confirmed adverse effects to fish reproduction from contaminant exposure and endocrine disruption, it is not known if there are behavior disorders or changes to reproductive capacity related to endocrine disruption and intersex characteristics observed in the Mississippi River sturgeon (Giesy *et al.* 2000 and Stewart *et al.* 2000).

The concentrations of PCB and organochlorine insecticide chemicals in the shovelnose sturgeon tissues from the affected area indicated that this group was exposed to more types of chemicals and at an apparently greater rate compared to the group from the reference site. PCB and the organochlorine insecticide chemicals detected in the sturgeon were widely used in the past, but have since been banned from use during the 1970s and early 1980s. PCB and the organochlorine insecticides such DDT and its chemical metabolites (DDD and DDE), chloradane and dieldrin remain persistent in the environment and bioaccumulate in fatty tissues.

Recommendations

The results of this screening level study will be used by program managers to help make a scientific and management decision to continue the investigation into advanced phases. We recommend that the investigation continue because of the weight of the evidence suggests that sturgeon from the Middle Mississippi River are stressed from chemical contamination and the information is needed for endangered species consultation activities.

Information on pallid sturgeon limiting factors is needed at this time to support ongoing Endangered Species Act Section 7 consultation with the Army Corps of Engineers over habitat modification actions in the Middle Mississippi River navigation system. The U.S. Fish and Wildlife Service has advised the Army Corps of Engineers that proposed changes to the commercial navigation system for the Mississippi River will jeopardize survival of the pallid sturgeon species.

The next phase of this investigation should be designed to confirm causal relationships between contaminant burden and health indices and to determine injuries to reproductive performance. A scope for a follow up study is outlined below for consideration in future planning.

1. Repeat the investigation in the affected area with larger sample size to allow for statistical correlation between selected health indices (endocrine disruption and histopathology) and contaminant exposure.

2. Survey reproductive performance (sperm count, egg count, fertile egg hatch success, egg-sac fry and larvae behavior and survival) of wild sturgeon from the affected area brought into captivity.

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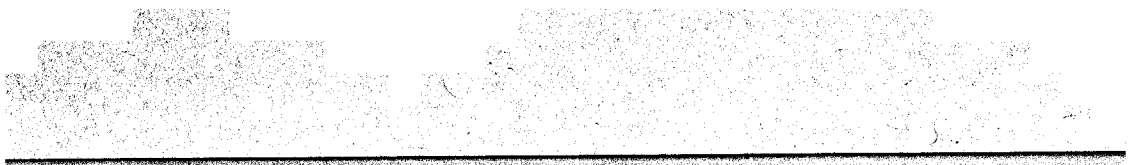
Summary of Federal Regulations for Taking Gray Wolves in the Eastern Distinct Population Segment
April 1, 2003

Gray wolves throughout the Eastern DPS are classified as "threatened" under the federal Endangered Species Act (ESA). However, different regulations apply to these threatened wolves, depending on the location of the animals within the Eastern DPS. Special regulations for Minnesota wolves have been in effect since 1978. Special regulations for the other Midwestern states took effect on April 1, 2003. There are no specific regulations for gray wolves in states that are east of Ohio, so the normal protections for threatened wildlife apply there (see 50 CFR 17.31).

All wolves killed or injured in the Eastern DPS must be reported to the U.S. Fish and Wildlife Service (FWS) or to the appropriate state or tribal conservation agency.

Situation	Special regulations for **Minnesota** wolves (see 50 CFR 17.40(d))	Special regulations for wolves in **other Midwestern states** - North Dakota, South Dakota, Nebraska, Kansas, Iowa, Missouri, Wisconsin, Illinois, Michigan, Indiana, & Ohio. DO NOT apply to states east of Ohio or to Minnesota (see 50 CFR 17.40(o))	Eastern Distinct Population Segment **states that are east of Ohio** - Pennsylvania, New Jersey, New York, Connecticut, Rhode Island, Massachusetts, Vermont, New Hampshire, & Maine. (see 50 CFR 17.31)
A. In defense of human life	Any person can kill or injure a wolf in defense of his/her life or the life of others	Any person can kill or injure a wolf in defense of his/her life or the life of others	Any person can kill or injure a wolf in defense of his/her life or the life of others
B. Protecting human safety	U.S. Fish and Wildlife Service (FWS) or its designated agents may remove wolves that are a "demonstrable but nonimmediate threat to human life or safety." (Not specifically included in 50 CFR 17.40(d), but included in Regional endangered species permit.)	Wolves that are a "demonstrable but nonimmediate threat to human life or safety" may be removed by FWS, other federal land management agencies, state or tribal conservation agencies, or designated agents of any of these agencies.	Wolves that are a "demonstrable but nonimmediate threat to human life or safety" may be removed by FWS, other federal land management agencies, state conservation agencies, or designated agents of any of these agencies.



Situation	Special regulations for **Minnesota** wolves (see 50 CFR 17.40(d))	Special regulations for wolves in **other Midwestern states** - North Dakota, South Dakota, Nebraska, Kansas, Iowa, Missouri, Wisconsin, Illinois, Michigan, Indiana, & Ohio. DO NOT apply to states east of Ohio or to Minnesota (see 50 CFR 17.40(o))	Eastern Distinct Population Segment **states that are east of Ohio** - Pennsylvania, New Jersey, New York, Connecticut, Rhode Island, Massachusetts, Vermont, New Hampshire, & Maine. (see 50 CFR 17.31)
C. Aiding a sick, injured, or orphaned wolf; disposing of a dead wolf; or salvaging for scientific study	May be done by FWS, other federal land management agencies, Minnesota Department of Natural Resources (DNR), or their agents	May be done by FWS, other federal land management agencies, state or tribal conservation agencies, or their agents	May be done by FWS, other federal land management agencies, state conservation agencies, or their agents
D. Salvaging a dead wolf for traditional cultural purposes by Native American tribes	Not included in 50 CFR 17.40(d), but may be done under permit issued by FWS; see G, below.	May be done by FWS, other federal land management agencies, state or tribal conservation agencies, or their agents	No specific provision under 50 CFR 17.31; may be done under permit issued by FWS; see G, below.
E. Removing wolves attacking lawfully present domestic animals	May be done by FWS and Minnesota DNR, or agents of these agencies	May be done by employees of FWS, state or tribal natural resource management agencies, or their agents	No specific provision under 50 CFR 17.31; may be done under permit issued by FWS; see G, below.
F. Taking wolves for research or conservation programs under ESA section 6 cooperative agreements	Minnesota DNR has full authority for such taking	State conservation agencies which have approved section 6 cooperative agreements with FWS have full authority for such taking	State conservation agencies which have approved section 6 cooperative agreements with FWS have full authority for such taking
G. Other forms of take may be carried our for various purposes under specific FWS permits, as authorized by 50 CFR 17.32	By various parties, if the take is for: • scientific purposes • enhancement of propagation or survival • zoological exhibition • educational purposes • incidental taking (with an HCP) • special purposes consistent with ESA	By various parties, if the take is for: • scientific purposes • enhancement of propagation or survival • zoological exhibition • educational purposes • incidental taking (with an HCP) • special purposes consistent with ESA	By various parties, if the take is for: • scientific purposes • enhancement of propagation or survival • zoological exhibition • educational purposes • incidental taking (with an HCP) • special purposes consistent with ESA